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DEPRESSION OF FOOD INTAKE INDUCED IN HEALTHY SUBJECTS BY GLUCAGON*

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ALTHOUGH most of the interest in glucagon has centered upon its effects on carbohydrate metabolism, there has also been evidence that it may play a direct part in the control of the human appetite. Schulman et al.[1] found that glucagon, given three times daily to a group of patients in a mental hospital, caused their food intake to diminish and their weight to decrease, and Stunkard, Van Itallie and Reiss[2] demonstrated that glucagon would abolish hunger contractions. Because Mayer[3] and other investigators[2] have indicated that hunger is suppressed at times when there is a large difference in the concentration of glucose in the blood of peripheral arteries and veins, indicating a rapid uptake of glucose by the peripheral tissues, any action of glucagon in depressing appetite or food intake has usually been ascribed to the fact that it produces a large arteriovenous difference in peripheral blood glucose concentration. Yet Bernstein and Grossman[4] have reported that experimentally elevating this arteriovenous difference by administering glucose did not affect the food intake of their experimental subjects. This has suggested that if glucagon does act to suppress food intake, its action must be based on some other mechanism than its effect on blood glucose. Therefore, the present studies were undertaken to explore the relation between glucagon, food intake, appetite and blood glucose. Both acute and chronic experiments were carried out.

ACUTE EXPERIMENTS

Methods

Subjects for the acute experiments were 8 healthy medical students of normal weight, each of whom volunteered to take part in ten to fifteen experiments. Each agreed to eat the same breakfast on each experimental day, to eat nothing else that day except what was offered at the test meals and to report any symptoms that he might experience during or after the experiment. Three experimental schedules were utilized, in a random, double-blind manner, as follows:

On "glucagon days" the subjects drank a disguised placebo solution, 200 ml. in volume, containing cyclamate,§ 13 ml. Ten minutes later, they were given glucagon, 1 mg. injected into the deltoid muscle.

On "glucose days" the subjects received an otherwise similar solution containing 100 gm. of glucose, and ten minutes later they received a placebo injection.

On "control days" the subjects drank the placebo solution and then had a placebo injection.

In all experiments appetizing test meals, of known caloric content, were presented thirty minutes, two hours or four hours after the injection. The subjects who had a test meal at thirty minutes received another meal at four hours. The first meal, served around noontime, consisted of a plate of small meat sandwiches, a quart of milk and a plate of cookies. The second meal, served around suppertime, consisted of a standard beef stew, a quart of milk and bread and butter. An excess of food was offered in each experiment. No subject was able to finish the entire amount. The subjects ate alone in standard, pleasant surroundings.

Blood samples were taken at zero time, thirty minutes, two hours and four hours; 5 ml. of venous blood was withdrawn from an antecubital vein while two 0.2-ml. samples were simultaneously taken from a finger puncture on the same side, the hand having been immersed previously in a water bath at 37°C. Blood glucose determinations were carried out in du-

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§In the form of Sucaryl, Abbott Laboratories, North Chicago, Illinois.

plicate by the method of Somogyi, as modified by Somogyi[5] and Nelson.[6]

Results

Typical experiments are shown in Figures 1-3. On the "control day" (Fig. 1) the subject received a placebo solution by mouth and a placebo injection.

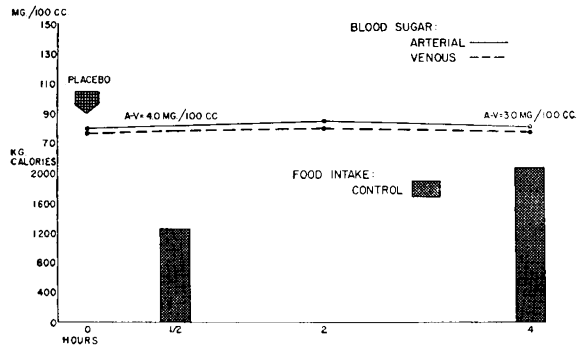

FIGURE 1. *Typical Experiment on the "Control Day."*

His blood glucose concentration remained low before the meal, and after the meal it returned to its original level within two hours. His intake was 1270 calories at the meal thirty minutes after the injection and 926 calories at the meal four hours after the injection.

The same subject, on a "glucose day" (Fig. 2), received 100 gm. of glucose and a placebo injection.

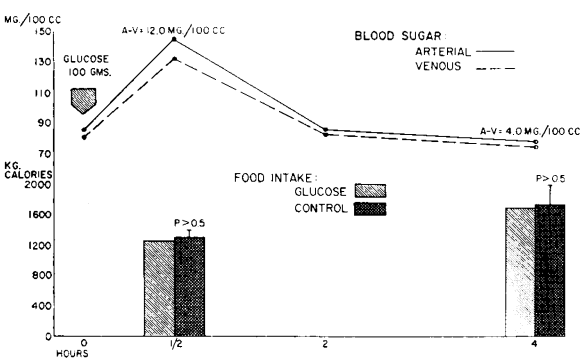

FIGURE 2. *Typical Experiment on the "Glucose Day."*

Thirty minutes later his blood glucose arteriovenous difference had risen to 14.1 mg. per 100 ml. At this time he ate 860 calories, an amount not significantly different from his mean control food intake of 816 calories. Four hours after the glucose injection, when his arteriovenous difference had returned to a low level, he took a second meal of 1175 calories. This also was not significantly different from his mean control caloric intake of 959 calories at the four-hour meals.

Figure 3 shows a "glucagon day" for this subject. Thirty minutes after the glucagon injection the arteriovenous difference had risen to 16.9 mg. per 100 ml., and he ate 773 calories (an amount not significantly different from the mean control intake of 816 calories at thirty minutes). However, four hours after the glucagon injection, when the arteriovenous glucose difference had returned to a postabsorptive level, he ate only 630 calories — significantly less than the mean control intake of 959 calories at four hours.

Only in experiments with glucagon was there a depression of food intake, which did not occur until the peripheral blood glucose concentration had returned to postabsorptive levels. This observation was repeated in many experiments.

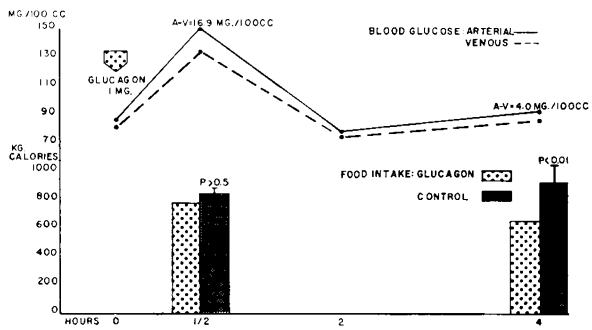

FIGURE 3. *Typical Experiment on the "Glucagon Day."*

Figure 4 represents composite blood glucose curves from all experiments with glucagon, glucose and placebo. The effects of 100 gm. of glucose given by mouth and of 1 mg. of glucagon, injected intramuscularly, upon the peripheral blood glucose concentration of these subjects were, in general, similar, and the mean arteriovenous differences at thirty minutes and at four hours were very much the same.

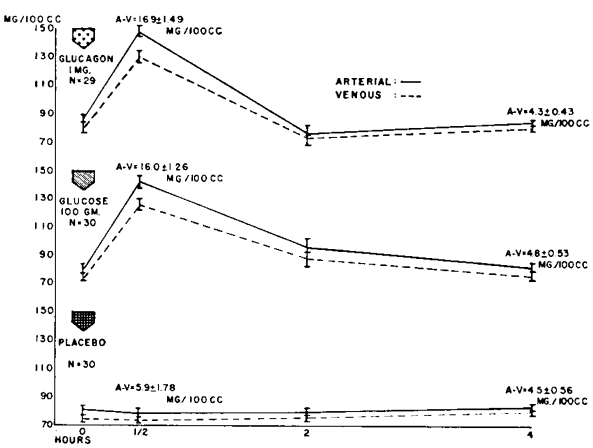

FIGURE 4. *Mean Arterial-Blood and Venous-Blood Glucose Curves.*

In spite of the fact that glucose and glucagon had similar effects on the arteriovenous difference, only glucagon depressed food intake (Fig. 5). Two hours after glucagon injection, the mean food intake of all subjects was a third less than the control values.

Four hours after glucagon, the mean food intake of all subjects was still decreased by 20 per cent. Glucose, by mouth, had no such effect. Furthermore, at half an hour, when both the blood glucose concentration and the arteriovenous difference were elevated, neither glucose nor glucagon reduced food intake. Glucagon alone affected food intake at test meals, and it did so only after blood glucose had returned to fasting levels.

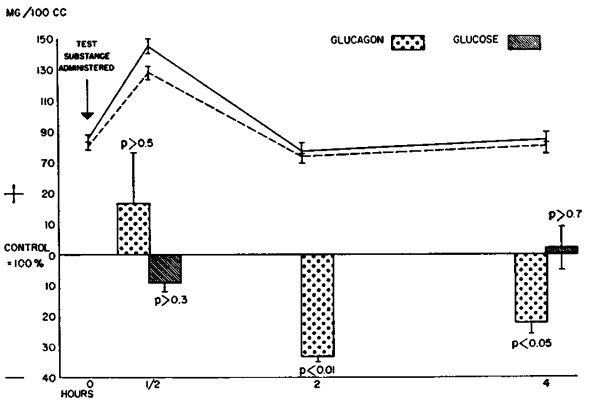

FIGURE 5. *Food Intake as a Percentage of the Control.*

The proportion of all experiments in which food intake was more than two standard deviations below the mean control level of the subject is shown in Figure 6. Glucagon produced a significant depression of food intake in 78 per cent of experiments at two hours and in 55 per cent of experiments at four hours, but it had no significant effect at half an hour. Thus,

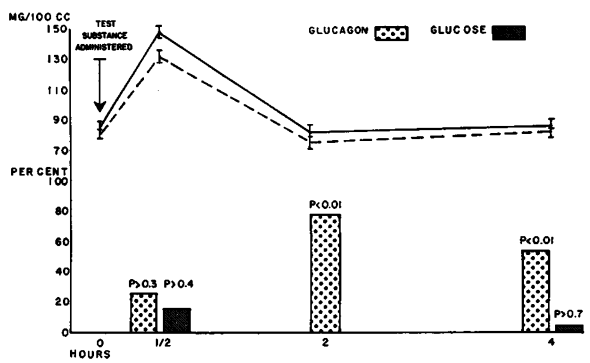

FIGURE 6. *Frequency of Depression of Food Intake (Expressed as a Percentage of All Trials).*

it is apparent that the effects of glucagon on food intake were delayed; they were of the greatest magnitude and were most consistently observed two hours after intramuscular injection.

In 15 of 29 experiments with glucagon, the subjects reported anorexia or slight nausea. These symptoms were of variable intensity and duration. They did not appear until at least ninety minutes after the glucagon injection, and they lasted for one to six hours thereafter. They were rarely so distressing as to modify the subjects' activities in any way. They reached maximum intensity shortly after their onset and then gradually diminished. Almost without exception these symptoms occurred in the presence of *fasting* blood glucose values.

Glucagon produced both anorexia and depression of food intake, but these two phenomena did not necessarily occur together (Fig. 7). Depression of food intake occurred without anorexia in 9 subjects, anorexia occurred without depression of food intake in 6, and they occurred together in 9. Thus, it appeared that the occurrence of anorexia recognized by the subjects was not a necessary prerequisite to the depression of food intake.

CHRONIC EXPERIMENTS

Methods

To ascertain the effects of long-term administration of glucagon on food intake, body weight and nitrogen balance, chronic experiments using as subjects 2 healthy medical students of normal weight were

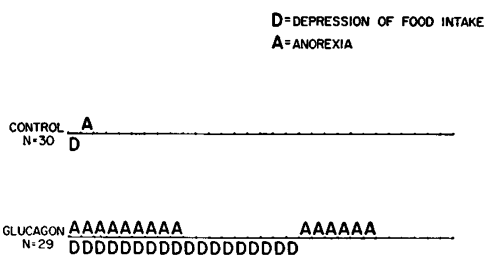

FIGURE 7. *Relation between Glucagon Administration, Anorexia and Depression of Food Intake.*

carried out. During a twenty-five-day period, these 2 men ate three meals per day in the laboratory, and a midnight snack also if they wished. They ate nothing outside the laboratory. During the first fifteen days of the experiment they were allowed a free choice of an appetizing menu, which included several varieties of meat, vegetables, potatoes, bread, fruit, ice cream, sandwiches, eggs and cereal. The amount that each man ate was determined solely by himself. Apart from the fact that he took his meals in the laboratory, his usual routine was not disturbed. The subjects were told that this was a metabolic experiment, and they were not aware that their appetite was being studied.

These subjects received, in a double-blind manner, either glucagon, 1 mg. intramuscularly three times a day, or a placebo injection. During the final six days of the experimental period each of the subjects received a diet identical with the diet that he had selected during the glucagon period, but he received no glucagon. They were weighed each morning under standard conditions.

Twenty-four-hour samples of urine were collected during the entire experiment and were analyzed for glucose, for nitrogen, with the use of the Koch–Mc-

Meekin[7] micromethod, and for creatinine by the method of Bonsnes and Taussky.[8]

Results

The food intake and body weight of 1 subject are shown in Figure 8. During an initial six-day control period, the mean caloric intake of the subject was 2904 calories, and he gained 0.5 kg. During the next six days, while he was receiving glucagon, his

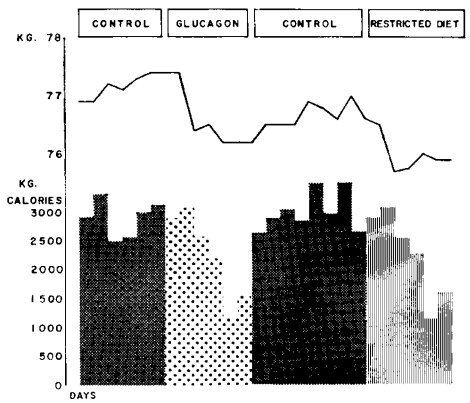

FIGURE 8. *Caloric Intake and Body Weight.*

daily intake dropped steadily and he lost 1.2 kg. The difference between the means for "glucagon" and "control" periods is significant at the 2 per cent level. During the second control period the mean daily caloric intake returned to 3006 calories, and he gained

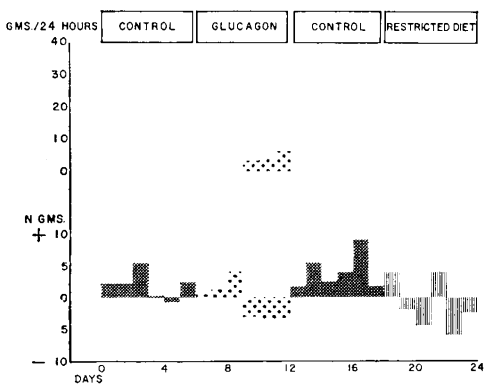

FIGURE 9. *Nitrogen Balance and Urinary Glucose.*

0.4 kg. During the last six days, when he was given a diet identical with the one that he had selected while he was receiving glucagon, he lost 0.7 kg. He reported neither nausea nor anorexia during the experimental period.

This subject consistently demonstrated a positive nitrogen balance during the control periods (Fig. 9), but a negative nitrogen balance developed on glucagon, especially during the last two days of the glucagon period. During the final experimental period, when he received a restricted diet but no glucagon, he had a negative nitrogen balance similar to that demonstrated when he received glucagon. The difference between the values for "control" and "glucagon" periods is significant at the 1 per cent level. The values for the "glucagon" and the "isocaloric" periods with no glucagon were not significantly different.

This subject had very little glycosuria. The daily glucose output did not exceed 6 gm. on any occasion, and the glycosuria appeared only during the last three days of glucagon administration.

In another subject similar though less dramatic effects on food intake and body weight were noted (Table 1). This man exhibited a marked glucosuria, the glucose excretion rising to approximately 40 gm. on the fourth day of glucagon administration, but there was no change in the volume of the urine and no significant ketosis at this time.

Discussion

Mayer[3] and others have found that when the difference in the concentration of glucose in the peripheral arteries and veins is elevated to a level

TABLE 1. *Effects on Weight and Nitrogen Balance.*

Days	Medication	Mean Kg. Cal./Day	Weight Change	Nitrogen Balance
			kg.	*gm.*
1-12	Control	2734	+0.8	+12.7
13-18	Glucagon	2249	−0.8	+5.9
19-24	Restricted diet	2249	−0.8	+11.8

of 15 mg. per 100 ml. or more by the administration of glucose, gastric contractions and the sensation of hunger are often suppressed. They have also found that gastric contractions and complaints of hunger usually begin at times when the peripheral arteriovenous glucose difference is 5 mg. per 100 ml. or less. Nevertheless, during the present experiment, the elevation of the blood glucose arteriovenous differences to levels of 15 mg. per 100 ml. or higher resulting from either glucose or glucagon had no significant effect upon the food intake at test meals offered to subjects when the arteriovenous difference was high. Whatever effect peripheral blood glucose levels may have had upon the appetite and hunger contractions, these did not appear to control the food intake.

On the other hand, the administration of glucagon intramuscularly regularly diminished the food intake of subjects who were offered an appetizing test meal two to four hours later. This depression of food intake was sometimes associated with symptoms of anorexia or with slight nausea, but often it was not, and sometimes such symptoms occurred even though food intake was not diminished. The effect of glucagon upon food intake took place at a time when the absolute values for blood glucose and the arteriovenous glucose difference had returned to postabsorptive levels. This effect did not occur during the

period immediately after glucagon administration, at a time when the blood glucose and arteriovenous differences were elevated, nor was it ever observed after the administration of glucose by mouth, even though this procedure produced comparable levels of blood glucose and comparable arteriovenous differences.

The action of glucagon upon food intake thus appeared to be independent of its effect upon the peripheral blood glucose concentration. The mechanism of the effect is not apparent. There were some clinical clues that gastrointestinal motility was inhibited at the time when symptoms of anorexia or nausea occurred, and, as has been stated, an acute inhibition of gastrointestinal motility has been shown to occur after the intravenous administration of glucagon to man.[2] Whether this is a local effect of glucagon upon the stomach or whether it is mediated through the central nervous system is not evident.

The chronic administration of glucagon appears to depress food intake and cause weight loss. Although a negative nitrogen balance and a moderate ketonuria may occur during the course of glucagon administration, our experiments suggest that these could be entirely explained on the basis of relative starvation produced by diminished food intake without the necessity of the postulation that glucagon has a direct catabolic action.

Recently, Unger et al.[9] have shown that the concentration of glucagon in the pancreatic veins of the dog varies inversely with the levels of the blood glucose. They have postulated that glucose is a hormone with a role in the regulation of blood glucose levels. If this is true, it is justifiable to speculate whether glucagon also has some role in the regulation of food intake in man. It is possible that glucagon and insulin, which oppose one another in the regulation of the blood glucose, also oppose one another in the regulation of food intake. Whereas insulin has the effect of depressing blood glucose levels and stimulating the hunger contractions, glucagon may have the opposite effect of elevating blood glucose levels and inhibiting hunger contractions. Bearing in mind that glucagon secretion appears to rise as the blood glucose concentration falls, one might speculate that its secretion begins gradually as the blood sugar returns to fasting levels, one to two hours after a meal, and that it may have the effect of somewhat ameliorating the hunger pangs that might otherwise occur during periods of prolonged fasting. This might provide a partial explanation for the commonly observed phenomenon that hunger rises to a peak at the time when meals are usually eaten, but if no food is then consumed, hunger pangs usually diminish somewhat with the passage of time.

Summary

With the use of a random double-blind procedure, 110 acute experiments were carried out with 8 healthy medical students to compare the effects of glucose, 100 gm. by mouth, glucagon, 1 mg. intramuscularly, and a placebo upon food intake.

Neither glucose nor glucagon had any significant effect upon the food intake of the subjects half an hour after administration, despite the fact that both produced significant elevations in the absolute blood glucose concentration and in the arteriovenous blood glucose difference at such times.

Glucagon, but not glucose or placebo, produced a significant reduction in the food intake at test meals. This effect was delayed, beginning one hour after administration, reaching a peak at two to three hours and continuing for five hours. The mean depression of caloric intake was 30 per cent below the mean control level.

The administration of glucagon occasionally caused moderate nausea and some complaints of anorexia, but there was no consistent relation between the occurrence of these symptoms and the depression of food intake.

Chronic experiments were carried out with 2 healthy subjects who were given either glucagon or a placebo three times a day during six-day periods, a double-blind procedure being used. During the glucagon period the subjects consistently ate less, lost weight, experienced a negative nitrogen balance and had some glycosuria.

Placing the experimental subjects on a food intake identical with that consumed while they received glucagon had similar metabolic effects even though glucagon was not administered. This suggested that the metabolic effects of glucagon, except the glycosuria, were secondary to diminished food intake.

A possible role of glucagon in the regulation of food intake in man is discussed.

We are indebted to Dr. W. R. Kirtley, of the Eli Lilly Company, for supplies of glucagon.

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